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19 November 93

TITLE OF INVENTION

A METHOD FOR PREVENTING OR CONTROLLING CATARACT

APPLICANT(S) FOR DO/EO/US

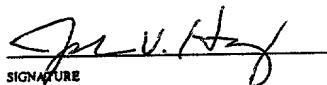
Johnston William McAvoy and Coral Gwenda Chamberlain

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. has been transmitted by the International Bureau.
 - c. is not required, as the application was filed in the United States Receiving Office (RO/US)
6. A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. have been transmitted by the International Bureau.
 - c. have not been made; however, the time limit for making such amendments has NOT expired.
 - d. have not been made and will not be made.
8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. A FIRST preliminary amendment.
 A SECOND or SUBSEQUENT preliminary amendment.
14. A substitute specification.
15. A change of power of attorney and/or address letter.
16. Other items or information:

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 08/648,092	INTERNATIONAL APPLICATION NO PCT/AU94/00694	ATTORNEY'S DOCKET NUMBER UNSYD 39709																
17. <input checked="" type="checkbox"/> The following fees are submitted:		CALCULATIONS																
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO..... \$ 880.00		PTO USE ONLY																
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Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)). \$																		
<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 15%; padding: 2px;">Claims</td> <td style="width: 15%; padding: 2px;">Number Filed</td> <td style="width: 15%; padding: 2px;">Number Extra</td> <td style="width: 15%; padding: 2px;">Rate</td> </tr> <tr> <td>Total Claims</td> <td>-20-</td> <td></td> <td>X \$22.00 \$</td> </tr> <tr> <td>Independent Claims</td> <td>- 3 -</td> <td></td> <td>X \$78.00 \$</td> </tr> <tr> <td colspan="2">Multiple dependent claims(s) (if applicable)</td> <td></td> <td>+ \$ 250.00 \$</td> </tr> </table>		Claims	Number Filed	Number Extra	Rate	Total Claims	-20-		X \$22.00 \$	Independent Claims	- 3 -		X \$78.00 \$	Multiple dependent claims(s) (if applicable)			+ \$ 250.00 \$	
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Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property + \$																		
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#2

This Certificate of Mailing by "Express Mail" is attached to the following documents in re:

Title: A METHOD FOR PREVENTING OR CONTROLLING CATARACT
Inventors: Application of Johnston William McAvoy and Coral
Gwenda Chamberlain

Serial No.: 08/648,092

Our Docket No. UNSYD 39709, including:

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Preston Jones

Date: June 21, 1996

A METHOD FOR PREVENTING OR CONTROLLING CATARACTTechnical Field

The present invention relates to a method for preventing or controlling pathological changes which occur in association with cataract formation in the mammalian eye by reducing the amount of or inhibiting the action of transforming growth factor-beta (TGF β). The invention also relates to the use of inhibitors of TGF β to prevent or minimise "aftercataract".

10

Background Art

Cataract is an opacity of the lens that interferes with vision. It is one of the most common of eye diseases and, though it may occur at any time in life, it often accompanies aging. In the USA, for example, up to 15 45% of people aged between 74 and 89 years suffer from cataract. Currently, the most commonly used treatment for cataract is surgical removal of the lens cells and subsequent implantation of a synthetic replacement lens within the remaining lens capsule. However, implantation 20 of a synthetic lens may only temporarily restore vision because residual cells associated with the lens capsule often grow rapidly and form new opacities. The latter condition is known as "aftercataract" or post-operative capsular opacification.

25 The TGF β family consists of a group of related proteins, the most extensively studied members being TGF β 1, TGF β 2 and TGF β 3 and it has been reported that these are all present in the eye.

Disclosure of the Invention

30 In one aspect, the present invention provides a method of preventing or controlling cataract or cataract-like disorders in the eye of a mammalian subject which comprises administering to the subject an effective amount of one or more inhibitors of TGF β .

35 Preferably, the mammalian subject is a human being

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but the present invention is also suitable for treating cataract or cataract-like disorders in other animals such as horses, cats, dogs or the like.

Typically, the inhibitors of TGF β are selected from 5 proteins, glycoproteins and proteoglycans.

Suitable proteins include antibodies, peptide "growth factors" such as FGF, or the like.

Suitable glycoproteins include α_2 -macroglobulin, laminin, collagen or the like.

10 Suitable proteoglycans include substances such as decorin, heparan sulfate proteoglycans, biglycan or the like.

In another aspect, the present invention provides an 15 ophthalmological formulation comprising one or more inhibitors of TGF β in a pharmaceutically acceptable carrier.

In a further aspect, the present invention provides 20 a method of preventing or controlling "aftercataract" formation in the eye of a mammalian subject following lens implant surgery which comprises implanting in the eye of the subject a lens coated with one or more TGF β inhibitors.

In yet another aspect, the present invention provides 25 a lens implant coated with one or more TGF β inhibitors.

In yet a further aspect, the present invention provides the use of inhibitors of TGF β in the manufacture of an ophthalmological formulation for preventing or controlling cataract or cataract-like disorders.

30 Brief Description of Drawings

Figure 1 shows phase contrast micrographs of lens epithelial explants from 21-day-old rats cultured with TGF β 2 and non-immune IgG (A,B) or with TGF β and anti-TGF β IgG (C,D). Explants were photographed after 3 days (A,C) 35 and 5 days (B,D) of culture.

Modes for Carrying Out the Invention

The biological activity of TGF β can be inhibited in a number of ways. One method of inhibiting the

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5 biological activity is by using an antibody directed against an active region of the TGF β molecule. TGF β biological activity can also be inhibited by the use of other molecules which sequester, inhibit or inactivate TGF β . For example, proteoglycans such as decorin act as specific TGF β -binding proteins.

10 The present inventors have shown that the aqueous and vitreous that surround the lens of the eye contain molecules that inhibit the cataract-changes induced in lens cells by TGF β and one or more of several inhibitory molecules mentioned above have been reported to be present in the ocular media.

15 The TGF β inhibitors can be administered according to the present invention either by topical application, by introduction into one or more chambers of the eye (for example, the anterior chamber), or as an intravenous injection at a site from which the inhibitors can be readily transported to the eye via the circulatory system. When the TGF β inhibitor is α_2 -macroglobulin, 20 this can also be administered by mouth or by some other suitable route other than by way of an ophthalmological preparation. For example, it may be possible to provide lens cells with elevated levels of α_2 -macroglobulin by administering a substance which causes an increase in serum levels of α_2 -macroglobulin by affecting its 25 synthesis or breakdown or enhancing its degree of transfer into the ocular media. Molecules related to α_2 -macroglobulin ie, derived from it or specifically designed to mimic its TGF β inhibitory properties but perhaps better able to pass through cellular membranes or 30 the gut, may be administered in topical applications or by mouth or by other route.

35 The effective amount of the inhibitors of TGF β required for use in the treatments according to present invention will vary with the inhibitor used, with the route of administration, the stage of condition under treatment and the host undergoing treatment, and is ultimately at the discretion of the physician.

Typically, the TGF β inhibitors are presented as a pharmaceutical or ophthalmological formulation. The treatment can be used as an adjunct to eye surgery to inhibit cataract-related changes that may occur as a 5 result of surgical intervention as, for example, in the formation of "aftercataract" following implantation of synthetic lens material. The present invention may also be suitable for treatment of individuals otherwise at greater than normal risk of cataract formation or of 10 being exposed to elevated TGF β levels near the lens.

Most of the inhibitors of TGF β mentioned above are commercially available.

Decorin and biglycan can be obtained by purification according to Choi et al. Note that PGI and PGII are 15 synonyms for biglycan and decorin respectively.

Heparan sulfate proteoglycans can be obtained according to the method of Yanagishita et al.

Ophthalmological formulations of the present invention are prepared according to conventional pharmaceutical formulating techniques. The carrier may 20 be of any form depending on the form of preparation desired for administration and the formulation may optionally contain other therapeutic ingredients. Typically, one or more inhibitors of TGF β can be included 25 in conventional irrigation solutions or viscoelastic solutions. Lens implants coated with one or more TGF β inhibitors may contain other therapeutic agents and may be prepared according to conventional techniques.

EXAMPLE 1.

30 Influence of TGF β alone and in combination with FGF on lens epithelial explants.

METHODS

Lens explants were prepared from both postnatal and adult rats and changes during 5 days culture with growth 35 factor(s) were monitored by light and electron microscopy, immunolocalisation of laminin, heparan sulphate proteoglycan and fibre-specific crystallins, and crystallin ELISAs.

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Each experiment involved culturing explants for up to 5 days without added growth factors (controls), with TGF β , with a combination of TGF β and FGF (TGF β /FGF), or with FGF alone. FGF is another growth factor that influences lens cell behaviour (Chamberlain and McAvoy, 1989; McAvoy et al., 1991). In some experiments, explants were prepared by a standard method used in our laboratory in which the adhering capsule serves as the substratum for the cells. In others, explants were inverted onto a laminin substratum. The latter method allows cell attachment, spreading and migration to be monitored as well as providing good visualisation of individual cells.

Bovine brain basic FGF was prepared and stored at -20°C as described by Chamberlain and McAvoy (1989). Ultrapure natural human TGF β 1 was obtained from Genzyme (Cambridge, MA) and stored at -80°C. Working stock solutions of TGF β and FGF were prepared (in culture medium or 1% bovine serum albumin-0.5 M NaCl in phosphate-buffered saline, respectively) and centrifuged at 10,000 g for 10 min at 4°C just before use.

Preparation and Culture of Lens Epithelial Explants: Standard Method

Eyes were removed from 10-day-old and 14-week-old Wistar rats under sterile conditions and placed in medium, that is, medium 199 containing bovine serum albumin and antibiotics as described by Hales et al (1992), pre-incubated at 37°C in 5% CO₂/air. Lenses were removed and incubated in 2 ml medium for 45-90 min (postnatal) or 1-2 hr (adult). Epithelia were then peeled away from fibres and pinned out with the cellular surface uppermost in culture dishes containing 2 ml medium as described by McAvoy and Fernon (1984). The whole epithelium was used, unless otherwise specified, and each dish contained 2-3 explants.

Approximately 3 hr after preparation of explants, medium was replaced (1 ml/dish) and 10 μ l samples of stock solutions of TGF β and/or FGF were added, as

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required, to give final concentrations of 20 and 40 ng/ml, respectively. Explants were cultured for 5 days with daily monitoring by phase contrast microscopy. At appropriate times explants were processed for light or 5 electron microscopy as described below. Alternatively, to assess the accumulation of fibre-specific crystallins, at the end of the culture period, explants were placed in 10 mM EDTA-0.02% Triton X-100, pH 10 (two explants in 200 μ l) and stored at -20°C, then used for β - and γ - 10 crystallin ELISAs with standards ranging from 0-20 ng/well.

Preparation and Culture of Lens Epithelial Explants: on Laminin Substratum

This method is as described by Hales et al (1992). 15 Briefly, on the day before the experiment, culture dishes were pre-coated with laminin. Whole explants were then prepared as described above, but with the cellular surface placed face down on the laminin and using lenses from 21-day-old rats; explants from rats of this age show 20 a strong migratory response to FGF (unpublished observation). Each dish contained three explants. Growth factor treatments and culture conditions were as described for standard explants, except that a lower concentration of FGF, 2 ng/ml, was used to ensure that 25 the main response to FGF alone was cellular migration rather than fibre differentiation. Responses were monitored daily by phase contrast microscopy.

Microscopy

Explants used for immunofluorescent localisation 30 were collected at the end of the culture period, fixed in Carnoy's fixative for 20 min at room temperature, transferred to 70% ethanol, then covered with a drop of melted 2.5% agar, before dehydrating in ethanol and embedding in paraffin. Sections were cut perpendicular 35 to the explant surface and stained with haematoxylin-phloxine or used for immunolocalisation of laminin, heparan sulphate proteoglycan (HSPG) or β - and γ - crystallins. For each antibody and each explant 20-30

sections cut through the central region were examined, and at least two explants were processed for each growth factor treatment. Controls for non-specific fluorescence were included routinely, that is, sections were treated 5 with non-immune rabbit serum instead of specific antibody. For whole mounts, explants were fixed in the culture dish with 100% ethanol and stained with haematoxylin-phloxine.

For ultrastructural studies, explants from 10-day-10 old rats were processed for transmission electron microscopy (TEM) and for scanning electron microscopy (SEM) as described by Lovicu and McAvoy (1992); explants were collected at 3 or 5 days of culture. Explants from adult rats were processed for SEM only at 5 days. For 15 both SEM and TEM, at least two explants were viewed for each treatment and, for TEM, 20-30 grids were viewed per explant.

RESULTS

Epithelial explants from postnatal rats (10 and 21 20 days old) were used for initial detailed studies. Because of the unusual nature of the observed responses to TGF β , a brief comparative study was then carried out using explants from adult rats.

Lens Explants from 10-day-old Rats: Standard Method

25 Phase contrast microscopy and SEM. In control and TGF β -treated explants the cells retained a characteristic epithelial cell morphology throughout the culture period, that is, they were present in a monolayer with cobblestone-like packing. In both cases, some cell debris 30 was detected on the monolayer surface. In TGF β -treated explants only, single cells or small groups of cells were also occasionally detected on the monolayer surface. SEM of explants cultured for 5 days showed that the apical surface of some cells in TGF β -treated explants overlapped 35 onto neighbouring cells.

TGF β /FGF- and FGF-treated explants were clearly distinguishable from controls within the first day of culture and indistinguishable from each other at this

stage. Cells were irregularly packed and intercellular spaces were common, an explant morphology that is generally associated with active cell migration (McAvoy and Chamberlain, 1989; McAvoy, 1988). After 2 days 5 culture some cells in the TGF β /FGF-treated, but not in the FGF-treated, explants were extensively elongated. The number of elongated cells varied between explants; they generally formed only a small proportion of the cellular population but because they often formed regular rows 10 they were quite distinct from the other cells in the explant which appeared similar to those in the FGF-treated explants. This marked difference between treatments was even more apparent at 3 days culture due to more cells becoming extensively elongated in TGF β /FGF- 15 treated explants. At this stage SEM showed that many of the elongated cells were attached to neighbouring cells at multiple sites along their length.

By 4 and 5 days culture, most of the cells in TGF β /FGF-treated explants were in multilayers and all 20 these explants had developed several regions where the cells were arranged in rosettes with elongated cells radiating out in a circular array from a focal point. Outside these rosettes, which occupied up to about 50% of the explant surface, there were some areas where similar 25 extensively elongated cells were arranged in parallel arrays. Remaining cells were less elongated and appeared irregularly arrayed as in FGF-treated explants.

SEM showed that, in regions outside the rosettes and 30 parallel arrays of extensively elongated cells, cells had numerous interlocking processes and appeared similar to the early differentiating fibres seen in explants treated with FGF alone. The morphological changes in explants from 10-day-old rats undergoing fibre differentiation in response to this concentration of FGF have been reported 35 in detail elsewhere (Lovicu and McAvoy, 1992); multilayering and the formation of numerous interlocking processes are well-established features of this process (Lovicu and McAvoy, 1992; Lovicu and McAvoy, 1989). In

the FGF/TGF β -treated explants, occasional patches of fibrillar extracellular matrix (ECM)-like material were noted on the explant surface. This matrix was dense and obscured the cells below.

5 **TEM.** Cells in explants cultured with FGF and TGF β /FGF for 5 days became multilayered and exhibited features of early fibre differentiation including elongation, sparse cytoplasmic organelles and nucleolar RNA particle aggregations; ball-and-socket joints typical of fibre
10 differentiation were also detected. Additionally in TGF β /FGF-treated explants, cells exhibiting margination of chromatin and cytoplasmic condensation were common, and membrane-bound cellular fragments and electron-dense bodies resembling secondary lysosomes were found within
15 many cells that otherwise appeared normal. These features are characteristic of apoptosis or programmed cell death (Wyllie et al. 1980; Williams et al., 1992). Similar apoptotic changes were also detected in TGF β /FGF-treated explants at 3 days.

20 Pockets of ECM-like granular material were commonly detected between cells (and sometimes appeared to be within cells) in TGF β /FGF-treated explants. Often near the cell membrane this material was present in a laminar arrangement and coated pits and vesicles were common in
25 such regions. Cells with prominent rough endoplasmic reticulum and Golgi, which also usually showed abundant arrays of microfilaments, were also found frequently in these explants.

30 In explants cultured with TGF β alone, the epithelial cells remained in a monolayer and were similar to controls except that, in the presence of TGF β , spaces were often present between cells. This, together with the overlapping of cells suggests that TGF β may be causing some disturbance of cell-cell interactions.

35 **Immunohistochemical localisation of laminin and HSPG.** The ECM molecules laminin and HSPG are both found in the normal lens capsule (Parmigiani and McAvoy, 1991; Mohan and Spiro, 1986) and, as expected, reactivity for both

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laminin and HSPG was detected in the capsule in all explants irrespective of treatment.

In TGF β /FGF-treated explants, reactivity for both laminin and HSPG was also localised within the explant in sites that were approximately similar in size and distribution to the pockets of ECM-like material seen by TEM. In FGF-treated explants, a few such regions were also detected; however, these were generally smaller and not as numerous as in explants treated with both growth factors. More sites exhibited reactivity for laminin than for HSPG and generally laminin reactivity was stronger.

In controls and TGF β -treated explants no pockets of reactivity for laminin or HSPG were detected within the cellular layer. Thus the intercellular spaces revealed by TEM in TGF β -treated explants did not contain ECM.

β -crystallin accumulation. To assess fibre differentiation we measured the fibre-specific β - and γ -crystallin content of explants at the end of the 5 day culture period by ELISA. Significant β -crystallin accumulation occurred only in explants cultured with TGF β /FGF or FGF ($P = 0.001$, compared with control); an apparent enhancement of β -crystallin accumulation in TGF β /FGF-treated explants relative to the FGF-treated explants did not reach statistical significance. None of the treatments induced significant accumulation of γ -crystallin within the 5 day culture period.

Complementary immunolocalisation studies confirmed these findings and revealed that β -crystallin appeared to be distributed throughout most cells in both TGF β /FGF- and FGF-treated explants.

Lens Explants from 21-day-old Rats: on Laminin Substratum

When explants were cultured cell surface down on a laminin substratum without growth factors, cells spread and migrated off the capsule onto the substratum forming an annulus around the explant. This process continued over the 5 day culture period and was significantly enhanced by FGF (Hales et al., 1992). The addition of TGF β , however, inhibited spreading and migration in the

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presence or absence of FGF so that a full annulus of cells did not develop; there were only a few isolated outgrowths of cells around the explant perimeter, and spreading and migration appeared to cease after 2 days of 5 culture. This is consistent with the observation that the cells at the leading edge of these outgrowths had few of the pseudopodia characteristic of rapidly migrating cells seen in FGF-treated explants at 2 days. There was no apparent difference between TGF β - and TGF β /FGF-treated 10 explants throughout the culture period.

During the first day of culture, all the cells in TGF β -treated explants (that is, with or without FGF) had a morphology very similar to those in controls; however, by day 2 most of the cells that had spread onto the 15 laminin substratum had become substantially elongated, some to the extent of being spindle-shaped or needle-like. In some regions cells that remained under the capsule also became elongated and aligned; these regions tended to extend between islands of epithelial-like 20 cells. By 3 days of culture, explants treated with TGF β mostly consisted of elongated cells and under the capsule differences between the peripheral and central regions of the explants became detectable. The periphery was well 25 populated with multilayers of aligned elongated cells, whereas cells in the central region were in reticular arrangements exposing regions of bare capsule.

Wrinkling of the capsule was noted in all explants cultured with TGF β under these explant conditions. The wrinkles had a reticular arrangement and were primarily 30 located in the central region of the explant. The wrinkles were most obvious at 2 days and generally became less pronounced during the remainder of the 5 day culture period.

Cell loss also appeared to be a major feature of 35 explants exposed to TGF β . Bare patches of capsule were initially detected in the central region of the explant at 3 days and condensed nuclei were readily visible in cells that had spread onto the laminin. Cell numbers then

progressively decreased and by 5 days the majority of the cells had been lost from the explant; the remaining cells retained the reticular arrangement first observed at 3 days.

5 Lens Explants from Adult Rats: Standard Method

Phase contrast and SEM. The morphological changes observed by phase contrast microscopy in these experiments were essentially similar to those reported for the explants from 21-day-old rats cultured on laminin, although as expected under these culture conditions no cells migrated off the capsule. Throughout the culture period there were no clear differences between TGF β - and TGF β /FGF-treated explants. During day 1, explants cultured with TGF β retained the cobblestone appearance characteristic of controls, but by 2 days many of the cells had elongated. Bare patches of capsule were detected at 3 days and these increased progressively during the culture period.

The latter finding was confirmed by SEM at 5 days which also revealed that the morphology of cells that remained in explants cultured with TGF β for 5 days was variable. Often cells were present in reticular arrays which seemed to consist mainly of mosaics of cells many of them epithelial-like. In other regions many cells were elongated and distinctly spindle- or needle-like and in some of these the cellular surface was covered with fine blebs. In the explant periphery, where more cells tended to survive, they were often present either as multilayers of smooth surfaced spindle-shaped cells or as more rounded cells with distinct surface blebbing typical of cells undergoing apoptotic cell death (Wyllie et al., 1980; Williams et al., 1992).

In FGF-treated explants, most cells retained an epithelial morphology although in the periphery some cells showed slight elongation characteristic of early fibre differentiation (Lovicu and McAvoy, 1992). Controls stayed as an epithelial monolayer throughout the culture period.

Immunohistochemical localisation studies. The pockets of laminin or HSPG reactivity reported above were not detected in explants from adult rats examined at the end of the culture period, irrespective of treatment.

5 Reactivity for β -crystallin was detected in some cells interspersed throughout the explant in controls and FGF-treated explants; in both TGF β and TGF β /FGF-treated explants the clumps of cells that survived for 5 days also included some cells that fluoresced for β -crystallin. No γ -crystallin was detected in any of the explants. There was thus no evidence that any of the 10 treatments stimulated ECM production or fibre-specific crystallin accumulation during the 5 day culture period.

SUMMARY

15 TGF β induced cells in explants to undergo an extensive and rapid elongation which had features that distinguished it from FGF-induced fibre differentiation. TGF β also induced accumulation of extracellular matrix, capsule wrinkling, cell death by apoptosis and 20 distinctive arrangements of cells. These TGF β -induced responses are characteristic of the changes reported to occur during formation of various types of cataracts (Novotny and Pau, 1984; Eshagian, 1982; Eshagian and Streeten, 1980; Green and McDonnell, 1985). Standard 25 explants from 10-day-old rats responded to TGF β only in the presence of FGF. Comparable explants from adult rats, or from 21-day-old rats cultured on a laminin substratum, responded readily to TGF β whether or not FGF was present.

EXAMPLE 2

Detailed description of an explant study using an antibody against TGF β to inhibit TGF β -induced cataract-like changes.

METHOD

35 Lens epithelial explants (2 per culture dish) were prepared from 21-day-old rats and trimmed to remove the peripheral region as described elsewhere (See Example 1, Standard Method). Explants were preincubated in culture

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medium at 37°C in 5% CO₂/air for approximately 3 hours before use.

A pan-specific polyclonal antibody against TGF β (rabbit IgG; British Bio-technology, Abingdon, UK; Cat. No. BDA 47,) was used; this neutralises TGF β 1, β 1.2, β 2, β 3, and β 5. This IgG and non-immune rabbit IgG were reconstituted in sterile phosphate-buffered saline to a concentration of 3 mg IgG/ml.

TGF β 2 (Genzyme, Cambridge, MA) was diluted with sterile medium to a concentration of 0.25 ng/10 μ l. Under sterile conditions, 33 μ l immune or non-immune IgG solution was mixed with 20 μ l TGF β 2 stock solution and 47 μ l medium, incubated at 37°C in 5% CO₂/air for 30 min, then diluted to 2 ml with medium. Preincubation medium was removed from two culture dishes and 1 ml TGF β -IgG mixture was added to each. All explants were cultured for 5 days with daily monitoring by phase contrast microscopy. Explants cultured with non-immune IgG served as controls for any effects of IgG itself on TGF β activity.

Figure 1 shows phase contrast micrographs of lens epithelial explants from 21-day-old rats cultured with TGF β 2 and non-immune IgG (A,B) or with TGF β and anti-TGF β IgG (C,D). Explants were photographed after 3 days (A,C) and 5 days (B,D) of culture. TGF β induces extensive elongation of cells (A, arrow); subsequently many cells are lost exposing regions of capsule which show wrinkles (B, arrow). Anti-TGF β completely blocks these changes and epithelial cells remain in a normal closely packed cobble-stone arrangement (C,D). The final concentrations of TGF β and IgG were 0.25 ng/ml and 50 μ g/ml, respectively.

RESULTS

In the presence of non-immune IgG, TGF β induced rapid elongation which occurred within 2-3 days (Fig. 1A) and by 5 days cells had been lost from the explant revealing wrinkling of the underlying capsule (Fig. 1B). These changes are typical of changes described in detail

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in Example 1 for explants cultured with TGF β in the absence of IgG.

In the presence of anti-TGF β , these changes were completely blocked. Throughout the 5 day culture period 5 the explants retained their original epithelial-like morphology (Fig. 1C, D) and were indistinguishable from explants cultured in medium alone.

EXAMPLE 3

10 Detailed description of an explant study using aqueous and vitreous to inhibit TGF β -induced cataract-like changes

METHOD

Aqueous and vitreous were obtained from the eyes of freshly slaughtered 2 to 3 year-old-cattle as follows. 15 Immediately on removal of the eye, the aqueous (about 1.5 ml) was collected using a sterile syringe fitted with a 23 gauge needle and an incision was made around the cornea to gain access to the lens. After carefully removing adhering iris, the lens was lifted out and the 20 vitreous adjacent to the lens, mainly liquid vitreous, was collected (2-3 ml) using a syringe without needle and taking care to avoid contamination with retina. The whole procedure was completed within about 1 hour of the death of the animals. Samples were transported to the 25 laboratory on ice and used as soon as possible.

Lens epithelial explants (2 per culture dish) were prepared from 21-day-old rats as described previously (See Example 1, Standard Method). Samples of aqueous or vitreous were diluted with an equal volume of sterile 30 culture medium (defined in Example 1), using repeated passage through a 23 gauge needle to ensure thorough mixing. These mixtures were equilibrated at 37°C for 30 minutes in 5% CO₂/air before use. Stock solutions containing 25 or 100 pg/10 μ l TGF β 2 (Genzyme, Cambridge, 35 MA) were prepared in sterile medium. Nine treatment groups were then set up by replacing medium in culture dishes containing explants with 1 ml medium or diluted aqueous or vitreous, with or without added TGF β , as

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indicated in Table 1. Explants were cultured and monitored for cataract-like changes by phase contrast microscopy. In particular, each explant was graded according to the extent of spindle-like elongation and 5 cell death, which are characteristically observed in explants cultured with TGF β (See Examples 1 and 2). Explants were photographed on day 4.

RESULTS

No significant changes were observed for any treatment on 10 day 1; explants retained typical epithelial morphology. Explants cultured with medium alone did not change throughout the culture period. With continuing culture, explants cultured with TGF β showed typical cataract-like changes, with more cell death for 100 pg/ml than 25 pg/ml 15 (Table 1). Aqueous virtually completely inhibited the effects of 25 pg/ml TGF β and partially inhibited the effects of 100 pg/ml. (With or without TGF β , aqueous also caused some shrivelling of the capsule.) Explants cultured with vitreous, with or without TGF β , showed 20 changes typical of early fibre differentiation (cf. Schulz et al., 1993), but there was no evidence of cataract-like changes; vitreous thus completely inhibited the cataract-like changes induced by TGF β .

25 Table 1. Inhibition of TGF β -induced cataract-like changes in rat lens epithelial explants by aqueous and vitreous

	Treatment	TGF β 2 concentration (pg/ml)		
		0	25	100
30	Day 3:			
	Culture medium	-	+++/††	++++/††
	Aqueous	-	-	++/††
	Vitreous	fd	fd	fd

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Day 4:

Culture medium	-	o/++++	o/++++
Aqueous	-	-	o/++++
Vitreous	fd	fd	fd

5 Day 5:

Culture medium	-	o/††††	o/†††††
Aqueous	-	-	o/†††††
Vitreous	fd	fd	fd

10 Explants were cultured with medium or with diluted aqueous or vitreous, with or without the addition of TGF β , as indicated. Four explants were subjected to each treatment. Code: -, negligible change; + - +++, indicates extent of spindle-like elongation; † - †††, indicates extent of cell loss; o, elongation assessment was invalidated by cell loss; fd, changes typical of early fibre differentiation with no cataract-like changes.

15

SIGNIFICANCE

This study indicates that the aqueous and vitreous that surround the lens of the eye contain molecules that inhibit the cataract-like changes induced in lens cells by TGF β . This effect may be due to the presence of one or more of several different kinds of inhibitory molecules which have been reported to be present in aqueous and vitreous: e.g. serum proteins, such as α_2 -macroglobulin; proteoglycans, such as decorin or heparan sulphate proteoglycans; or other peptide 'growth factors' such as FGF. All these molecules have been reported to bind to and/or inhibit TGF β activity (LaMarre et al., 1991; Yamaguchi et al., 1992; McCaffrey et al., 1992; Hales et al, in press). α_2 -macroglobulin is synthesised by the cornea (Twining et al. 1994) and is present in the aqueous (Ando et al., 1993); it probably enters the aqueous and vitreous along with other serum proteins found in these media (see, for example, Beebe et al., 1986). It has been shown that decorin is present near the

lens in proliferative vitreoretinopathy (Hagedorn et al., 1993). Heparan sulphate is present in the vitreous, probably in association with extracellular matrix proteoglycans (Kamei et al., 1992). FGF is reported to be 5 present in vitreous, and in much lower amounts in aqueous (Schulz et al., 1993), and to suppress the formation of TGF β -induced spindle-cell formation in lens explants (Hales et al, in press). Other molecules known to bind to 10 TGF β and/or inhibit its activity, which may be contributing to the observed inhibitory effects of aqueous and/or vitreous, include biglycan (Yamuguchi et al., 1990), laminin and collagen (Paralkar et al., 1991).

EXAMPLE 4

15 Detailed description of an explant study using α_2 -macroglobulin to inhibit TGF β -induced cataract-like changes

METHOD

α_2 -macroglobulin prepared from bovine plasma was obtained from Boehringer Mannheim Australia (Castle Hill, NSW; 20 #602 442). Lens epithelial explants (2 per culture dish) were prepared from 21-day-old rats as described previously (See Example 1, Standard Method). α_2 -macroglobulin was dissolved in culture medium (defined in Example 1) at a final concentration of 400 μ g/ml. The 25 solution was sterilised by passing through a 0.22 μ m filter and a portion was diluted with an equal volume of sterile medium. These solutions were equilibrated at 37°C in 5% CO₂/air before use. A stock solution containing 25 pg/10 μ l TGF β 2 (Genzyme, Cambridge, MA) was prepared in 30 sterile medium. Six treatment groups were then set up by replacing medium in culture dishes containing explants with 1 ml medium or 1 ml medium containing 200 or 400 μ g/ml α_2 -macroglobulin, with or without added TGF β , as indicated in Table 2. Explants were cultured and 35 monitored for cataract-like changes by phase contrast microscopy. In particular, each explant was graded according to the extent of spindle-like elongation and cell death, which are characteristically observed in

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explants cultured with $\text{TGF}\beta$ (See Examples 1 and 2). Explants were photographed on days 3-5.

Table 2. Inhibition of $\text{TGF}\beta$ -induced cataract-like changes in rat lens epithelial explants by α_2 -macroglobulin

5

	Treatment	TGF β 2 concentration (pg/ml)	
		0	25
<i>Day 2:</i>			
10	Culture medium	-	+
	α_2 -MG, 200 $\mu\text{g}/\text{ml}$	-	-
	α_2 -MG, 400 $\mu\text{g}/\text{ml}$	-	-
<i>Day 3:</i>			
15	Culture medium	-	+++/†
	α_2 -MG, 200 $\mu\text{g}/\text{ml}$	-	+
	α_2 -MG, 400 $\mu\text{g}/\text{ml}$	-	+
<i>Day 4:</i>			
20	Culture medium	-	○/††††
	α_2 -MG, 200 $\mu\text{g}/\text{ml}$	-	+/†
	α_2 -MG, 400 $\mu\text{g}/\text{ml}$	-	+/†
<i>Day 5:</i>			
	Culture medium	-	○/††††
	α_2 -MG, 200 $\mu\text{g}/\text{ml}$	-	-/†
	α_2 -MG, 400 $\mu\text{g}/\text{ml}$	-	-/†
25			
25	Explants were cultured with medium, with or without the addition of α_2 -macroglobulin (α_2 -MG) and/or $\text{TGF}\beta$, as indicated. Four explants were subjected to each treatment. Code: -, negligible change or reverted to predominantly epithelial morphology; + - +++, indicates extent of spindle-like elongation; † - †††, indicates extent of cell loss; ○, elongation assessment		
30			

was invalidated by cell loss.

RESULTS

No significant changes were observed in explants cultured with medium alone or with medium containing α_2 -5 macroglobulin; the explants retained typical epithelial morphology throughout the culture period. On days 2-5, explants cultured with TGF β showed typical cataract-like changes (Table 2). The TGF β -induced changes were substantially inhibited by including α_2 -macroglobulin in 10 the medium.

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Article 31
Claims

1. A method of preventing or controlling cataract or cataract-like disorders in the eye of a mammalian subject which comprises administering to the subject an effective amount of one or more inhibitors of TGF β .
5
2. A method according to claim 1 wherein the one or more inhibitors of TGF β are selected from proteins, glycoproteins and proteoglycans.
3. A method according to claim 2 wherein the protein 10 inhibitors of TGF β are selected from antibodies and peptide growth factors.
4. A method according to claim 2 wherein the glycoprotein inhibitors of TGF β are selected from α_2 -macroglobulin, laminin and collagen.
- 15 5. A method according to claim 2 wherein the proteoglycan inhibitors of TGF β are selected from decorin, heparan sulfate proteoglycans and biglycan.
6. An ophthalmological formulation comprising one or 20 more inhibitors of TGF β in an ophthalmologically acceptable carrier but excluding conventional pharmaceutically acceptable carriers.
7. An ophthalmological formulation according to claim 6 wherein the inhibitors of TGF β are as defined in claim 2, 3, 4 or 5.
- 25 8. A method of preventing or controlling "aftercataract" formation in the eye of a mammalian subject following lens implant surgery which comprises implanting in the eye of the subject a lens coated with one or more TGF β inhibitors.
9. A method according to claim 8 wherein the TGF β 30 inhibitors are as defined in claim 2, 3, 4 or 5.
10. A lens implant coated with one or more TGF β inhibitors.
11. A lens implant according to claim 10 coated with one 35 or more TGF β inhibitors as defined in claim 2, 3, 4 or 5.
12. The use of inhibitors of TGF β in the manufacture of an ophthalmological formulation for preventing or controlling cataract or cataract-like disorders.

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13. Use according to claim 12 wherein the TGF β inhibitors are as defined in claim 2, 3, 4 or 5.

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<p>(21) International Application Number: PCT/AU94/00694</p> <p>(22) International Filing Date: 11 November 1994 (11.11.94)</p> <p>(30) Priority Data: PM 2540 19 November 1993 (19.11.93) AU</p> <p>(71) Applicant (<i>for all designated States except US</i>): THE UNIVERSITY OF SYDNEY [AU/AU]; Parramatta Road, Sydney, NSW 2006 (AU).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (<i>for US only</i>): McAVOY, Johnston, William [AU/AU]; 20 Warwick Street, Stanmore, NSW 2048 (AU). CHAMBERLAIN, Coral, Gwenda [AU/AU]; 31/26 Charles Street, Five Dock, NSW 2046 (AU).</p> <p>(74) Agent: GRIFFITH HACK & CO.; GPO Box 4164, Sydney, NSW 2001 (AU).</p>		<p>(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).</p> <p>Published <i>With international search report.</i></p>	
<p>(54) Title: A METHOD FOR PREVENTING OR CONTROLLING CATARACT</p> <p>(57) Abstract</p> <p>The present invention relates to a method for preventing or controlling pathological changes which occur in association with cataract formation in the mammalian eye by reducing the amount of or inhibiting the action of transforming growth factor-beta (TGFβ). The invention also relates to the use of inhibitors of TGFβ to prevent or minimise "aftercataract".</p>			

PTO 21 JUN 1996

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
DESIGNATED/ELECTED OFFICE (DO/EO/US)

IN RE THE APPLICATION OF:)
Johnston William McAvoy)
and Coral Gwenda Chamberlain)
)
SERIAL NO.: 08/648,092) Docket No.: UNSYD 39709
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FILED: May 17, 1996)
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INTERNATIONAL APPLICATION)
NO. PCT/AU94/00694)
)
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)
PRIORITY DATE:)
19 November 1993) June 21, 1996
) Los Angeles, CA 90024
FOR: A METHOD FOR)
PREVENTING OR)
CONTROLLING CATARACT)

)

TRANSMITTAL OF DECLARATION AND POWER OF ATTORNEY

Hon. Commissioner of Patents and Trademarks
BOX PCT
Washington, D.C. 20231

Attention: DO/EO/US

Dear Sir:

To complete the necessary requirements for entering the National Stage in the United States of America as an Elected Office, Applicants submit herewith an executed Declaration and Power of Attorney for the above-identified application. The Declaration and Power of

Serial No. 08/648,092

Attorney is being filed prior to notification of deficiency by the Office. Accordingly, it is believed that no fee is due in connection with this paper.

Please charge any additional fees in this matter to our Deposit Account No. 06-2425. A duplicate copy of this paper is enclosed.

Respectfully submitted,

FULWIDER PATTON LEE & UTECHT, LLP

By: John V. Hanley
John V. Hanley
Registration No. 38,171

JVH:law
Enclosures
10877 Wilshire Blvd., Tenth Floor
Los Angeles, California 90024
Tel.: (310) 824-5555
Fax: (310) 824-9696

DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION

As the below named inventors, we hereby declare that:

Our residences, post office addresses and citizenships are as stated below next to our names,

We believe we are original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled **A METHOD FOR PREVENTING OR CONTROLLING CATARACT** the specification of which (check one)

 is attached hereto
XX was filed on May 17, 1996
Application Serial No.

and was amended on May 17, 1996 and May 22, 1996
(if applicable)

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

We acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Sec. 1.56(a).

We hereby claim foreign priority benefits under Title 35, United States Code, Sec. 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)	Priority Claimed
PM2540 <u>Australia</u> <u>19 November 1993</u>	<u>X</u> <u>Yes</u> <u>No</u>
Number Country Day/Month/Year filed	

We hereby claim the benefit under Title 35, United States Code, Sec. 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Sec. 112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Sec. 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

Appln. Serial No.	Filing Date	Status (patented, pending abandoned)
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We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

We hereby appoint the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

RICHARD A. BARDIN, Registration No. 20,365, GILBERT G. KOVELMAN, Registration No. 19,552, JOHN S. NAGY, Registration No. 30,664, CRAIG B. BAILEY, Registration No. 28,786, RONALD E. PEREZ, Registration No. 36,891 and JOHN V. HANLEY, Registration No. 38,171. Direct all telephone calls to John V. Hanley at telephone No. (310) 824-5555.

Address all correspondence to:

FULWIDER, PATTON, LEE & UTECHT
10877 Wilshire Boulevard
Tenth Floor
Los Angeles, California 90024

Full name of first inventor: JOHNSTON WILLIAM McAVOY

Inventor's signature: W McAvoy

Date: 5th JUNE, 1996

Residence: 20 Warwick Street
Stanmore NSW 2048
Australia AUX

Citizenship: Australian

Post Office Address: 20 Warwick Street
Stanmore NSW 2048
Australia

Full name of second inventor: CORAL GWENDA CHAMBERLAIN

Inventor's signature: G Chamberlain

Date: 5th JUNE, 1996

Residence: 31/26 Charles Street
Five Dock NSW 2046
Australia AUX

Citizenship: Australian

Post Office Address: 31/26 Charles Street
Five Dock NSW 2046
Australia